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Experimental soil warming and cooling alters the partitioning of recent assimilates: evidence from a ^{14}C-labelling study at the alpine treeline

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Abstract

Despite concerns about climate change-effects on ecosystems functioning, only little is known on how plant assimilate partitioning changes with temperature. Particularly large temperature-effects might occur in cold ecosystems where critical processes are at their temperature limit. In this study, we tested temperature effects on carbon (C) assimilate partitioning in a field experiment at the alpine treeline. We warmed and cooled soils of microcosms planted with *Pinus mugo* or *Leucanthemopsis alpina,* achieving daily mean soil temperatures (3-10 cm depth) around 5.8, 12.7 and 19.2°C in cooled, control and warmed soils. We pulse-labelled these systems with $^{14}CO_2$ for one photoperiod and traced ^{14}C over the successive four days. Plant net ^{14}C uptake increased steadily with soil temperature. However, ^{14}C amounts in fungal hyphae, soil microbial biomass, soil organic matter, and soil respiration showed a non-linear response to temperature. This non-linear pattern was particularly pronounced in *P. mugo*, with five times higher ^{14}C activities in cooled compared to control soils, but no difference between warmed and control soil. Autoradiographic analysis of the spatial distribution of ^{14}C in soils indicated that temperature effects on the vertical label distribution within soils depended on plant species. Our results show that plant growth, in particular root metabolism, is limited by low soil temperature. As a consequence, positive temperature effects on net C uptake may not be paralleled by similar changes in rhizodeposition. This has important implications for predictions of soil C storage, because rhizodeposits and plant biomass strongly vary in their residence time.

Introduction

Global mean temperatures have increased by almost 1°C since pre-industrial times, most likely as a consequence of anthropogenic activities. Warming is expected to continue in this century, with the largest temperature increases in cold regions, i.e. at high altitudes and at high latitudes (Bradley et al. 2004; Rebetez and Reinhard, 2007; IPCC, 2013). Many physiological processes are limited by low temperatures. Therefore, ecosystem responses to warming, including carbon (C) cycling may be particularly pronounced in cold regions (Rustad *et al.* 2001; Aerts, Cornelissen & Dorrepaal 2006). Despite ongoing research, our capacity to predict climate warming effects on C cycling are limited because an integrated understanding of the mechanisms involved is lacking (Chapin *et al.* 2009; Bahn *et al.* 2010).

Understanding ecosystem-level responses to warming is challenging, partly because individual processes constituting the ecosystem's C cycle are coupled and differ in their temperature sensitivity. For example, plant photosynthesis is strongly controlled by photon flux and thus relatively independent of temperature. Sunlit leaves can therefore maintain positive (although low) rates of photosynthesis even at 0°C air temperature (e.g. Kolari et al. 2007). In contrast, soil respiration responds more strongly to warming, with the soil CO_2 efflux increasing exponentially with soil temperature (e.g. Kirschbaum, 1995). Yet another pattern is found for plant growth. Several studies indicate a discontinuous temperature dependency of root growth and wood formation, which appear to require a minimum (threshold) temperature of 6°C (Körner 1998; Alvarez-Uria and Körner 2007; Rossi et al. 2007).

Another uncertainty is that temperature varies among ecosystem compartments. Especially in forests, canopies very effectively shield soils from solar radiation, therefore decoupling above- and belowground temperatures. Because tree roots appear to require a minimum soil temperature of 6°C to grow (Alvarez-Uria & Körner 2007) and because soil cools with canopy expansion,

closed forest fails to establish in cold environments (Körner 1998). This mechanism has been suggested to determine the altitudinal and latitudinal formation of distinct treelines (Körner & Paulsen 2004).

Given the interdependency of processes and their different temperature sensitivities, warming will not only alter C cycling rates but also C allocation and partitioning among different C pools. A large fraction of soil C inputs occur via rhizodeposition (i.e. root exudation and root litter production), which is tightly linked to belowground C allocation. A critical question is therefore how warming affects soil C inputs and the partitioning of these inputs among soil microbial biomass and soil organic matter fractions differing in characteristics including turnover rates (Chapin *et al.* 2009; Bahn *et al.* 2010; Kuzyakov & Gavrichkova 2010). Across latitudinal gradients, aboveground plant productivity and temperature are correlated (Vogel *et al.* 2008). At the same time, the fraction of C allocated belowground decreases with productivity (Litton, Raich & Ryan 2007) and with mean annual temperatures (Vogel *et al.* 2008; Kane & Vogel 2009). This shift in partitioning among plant compartments might be a response to reduced nutrient availability in cold condition (Giardina *et al.* 2003; Litton *et al.* 2007). Similar dependencies of allocation on temperature have been found under simulated warming with a proportional increase of C allocated to wood and branches and a decrease in allocation to belowground biomass (Savage *et al.* 2013). However, most of these studies were carried out in temperate ecosystems, and little is known about the temperature-dependency of C allocation near the temperature threshold for root growth. For example, many plant species are associated with mycorrhizal fungi, and it remains to be tested whether these symbionts could functionally substitute roots when low temperatures limit root growth (cf. Pumpanen et al. 2012). Such a mechanism could be possible if plants are C sink- (i.e. root growth) rather than source-limited (i.e. photosynthesis) at low temperatures.

Here, we present a study of C dynamics in microcosms subjected to experimental soil warming and cooling in the field near the alpine treeline. Individuals of a woody and an herbaceous species were pulse-labelled with $^{14}CO_2$ and the label traced throughout the plant-soil systems. Specifically, we tested for a soil temperature effect on (1) C partitioning between above- and belowground plant organs, (2) transfer of recently assimilated C to soil organic matter and microbial biomass, and (3) cycling rates of the label through the plant-soil system. Our temperature manipulations covered the soil temperature at the growth limit of trees. We therefore also checked whether (4) the parameters investigated change linearly with soil temperature, or whether non-linear phenomena (e.g. threshold responses) occurred. Although not the primary objective of our study, we further were interested whether responses differ between species.

Materials and Methods

Experimental design

We studied C cycling and partitioning of new assimilates among pools in a field experiment in the Swiss Central Alps at Stillberg (46°46'20" N, 9°51'56" E, 2280 m a.s.l., ≈200 m above the local treeline; see Hagedorn et al. 2010 for details). We manipulated soil temperature in microcosms containing the tree species *Pinus mugo* subsp. *mugo* (Turra) or the forb species *Leucanthemopsis alpina* (L., Heywood). Both species frequently occur at our study site and are quite typical for this type of environment. Microcosms were made from 10 cm diameter×10 cm length polyethylene tube sections that were closed at the bottom with a 2 mm nylon mesh. Microcosms were filled with a mor-type organic layer of a Norway spruce forest (Podzol on non-carbonated sandstone, 1500 m a.s.l.) sieved through a 0.5 cm mesh to remove stones and large roots. The soil was characterised by the following properties: pH ($CaCl_2$) = 2.8, soil organic C = 48.2%, N = 1.7%, C : N ratio = 29.1 (Walthert *et al.* 2003; Pannatier-Graf *et al.* 2011). In each

microcosm, we planted either a three-year old *P. mugo* tree, or a three-month old *L. alpina* individual. Both plant species had been grown from seeds collected in the Swiss Alps at altitudes of 2000–2200 m a.s.l.. Pine saplings were about 8-10 cm tall when set in the pots. In addition, each microcosm contained a mesh bag of 3 cm diameter×8 cm length to assess fungal hyphae ingrowth (50 µm nylon mesh, filled with 50 g C-free quartz sand to allow quantitative extraction of hyphae; adapted from Wallander et al. 2001).

At the end of June 2012, shortly after snowmelt, six blocks of 100×50 cm were excavated to a depth of 15 cm and the circumference of each block insulated with 2 cm thick styrofoam boards. Each block was divided into three plots of 33×50 cm each, using the same 2 cm styrofoam insulation. Soil temperature treatments were randomly assigned to plots, with soil temperature being either increased (warming treatment), reduced (cooling treatment), or left unmanipulated (control treatment). The temperature manipulation aimed at cooling or warming soils by about 6°C over the top 10 cm. Warming was achieved with soil heating cables (HD5034, Thermoforce, Cockermouth, Cumbria, UK) spread at the bottom of the plots with 8 cm distance between loops. The electric power deployed in the treatment period was 8.8 W m^{-2}. Cooling was achieved with silicone tubing through which an aqueous anti-freeze solution was circulated using a 20 l cooling bath set at a temperature of -2.5°C (K20-mpc-NR, Huber Kältemaschinenbau GmbH, Germany). Both heating cables and cooling tubing were covered with 2 cm of soil. In the control plots, soil was added to the same level as the treated plots, but remained free of cables and tubing. Six microcosms per plot were arranged on a 2×3 grid, with each plant species being surrounded by neighbours of the other species. The space between the pots was filled with the previously excavated soil material. Finally, the ground was covered with larch litter to protect the bare soil and limit direct warming by solar radiation. To prevent rodent damage, the experiment was enclosed with an electric fence. Soil temperatures were recorded hourly in one randomly selected

pot×treatment×block combination, using automatic data loggers installed at 3, 7, and 10 cm depth (DS1922L, Maxim Integrated, San Jose, CA). Air temperature and precipitation were recorded by the climate station present at the site.

Overall, the experimental design comprised 18 plots for the temperature treatment (6 block×3 soil temperature treatments). Each plot contained three pots per plant species, resulting in a total of 108 pots. Of these three pots, which were pseudoreplicates with respect to the temperature treatment, two were harvested destructively, while the remaining one was used to analyse the ^{14}C distribution using an autoradiographic technique. On June 21, 2012, the soil temperature manipulation was started. Until radiolabelling, soil CO_2 efflux was measured weekly, simultaneously with volumetric soil water content (HH1 soil moisture probe, Delta-T, Burwell, UK) and soil temperature (at 1, 4, 7 and 10 cm depth). Because August 2012 was very warm and dry, we irrigated plots with 4 l m^{-2} on August 13. Climatic conditions during experiment are shown in Fig. 1. Long term annual precipitation and air temperature at the site average around 1100–1200 mm and 2.1°C, respectively (1975–2009, Dawes et al. 2013).

^{14}C pulse labelling

Starting on August 10, the microcosms were labelled block-wise, and soil CO_2 and $^{14}CO_2$ evolution tracked on a daily basis. For the application of the ^{14}C-label, an additional block was set up. This time, however, each plot consisted of a plastic box filled with quartz sand into which the heating cables and cooling tubing were installed. As in the regular field setup, the boxes were insulated laterally with styrofoam. The microcosms were transferred block-wise into the respective plastic boxes, and the entire setup closed with an air-tight acrylic chamber (106×44 cm ground area×56 cm height). A water-filled rim surrounding all plastic boxes served as air-tight seal. Two heat exchangers fitted with three fans each were installed inside the acrylic chamber,

allowing to keep the air temperature below 20°C by manually adjusting the flow from a cooling bath. Treatment position during the labelling was re-randomized for every block by switching the three boxes before every labelling. In summary, this set-up achieved different soil temperatures for each plot, but equal aboveground conditions (temperature, humidity, CO_2 concentration, and ^{14}C activity) since the plots shared a common headspace with forced air circulation.

During the ^{14}C labelling, a pump was circulating air from the chamber's headspace through an infra-red gas analyser (LI-6200, Licor, Lincoln, NE), then through a glass bulb containing $Na_2{}^{14}CO_3$ solution, and then back into the chamber. $^{14}CO_2$ was released by adding diluted sulphuric acid to the $Na_2{}^{14}CO_3$ solution through a septum port. CO_2 concentrations in the chamber headspace were kept between 300 and 500 ppm by releasing CO_2 from unlabelled Na_2CO_3. Microcosms designed for destructive analyses and for autoradiographic imaging were labelled separately, on different days. Per microcosms, 50 or 650 kBq ^{14}C were released for destructive harvest or autoradiographic analysis, respectively. The labelling chamber was kept closed until 18:00 to maximize $^{14}CO_2$ uptake. Then, the microcosms were sealed at the bottom with a thin plastic film and transferred back to their original block. The net amount of $^{14}CO_2$ assimilated during the labelling photoperiod was determined by sampling 300 ml of headspace air with a syringe in the morning, just after releasing the $^{14}CO_2$, and again in the evening, before opening the chamber.

Microcosms designed for plant and soil analyses were harvested five days after start of the labelling (4½ days after the labelling chamber was removed). Half of the microcosms designed for soil autoradiographic analysis were harvested two days after labelling, the other half three days later (i.e. five days after labelling).

Soil respiration and dissolved organic matter

Soil CO_2 and $^{14}CO_2$ efflux were determined using a micro-chamber (a 2.7 cm diameter test tube cut to a length of 6 cm and inserted 3 cm into the soil). Over the 7 weeks preceding the radio-labelling, soil respiration was determined weekly by measuring the CO_2 increase over 60 seconds in a closed system consisting of a membrane pump and a LI-820 CO_2 analyser (LICOR, Lincoln, NE) connected to the micro-chamber. Soil temperature and moisture were recorded simultaneously. During and following the labelling, soil respiration was trapped in a vial containing 2 ml of 1 M NaOH placed inside the closed micro-chamber. The NaOH solution was replaced every 24 hours and analysed later for CO_2 and $^{14}CO_2$. CO_2 was quantified by acid titration of a 0.9 ml aliquot with 0.04 M HCl after precipitation of carbonates with 1.0 ml 0.5 M $BaCl_2$. Trapped $^{14}CO_2$ activity was determined by liquid scintillation counting of a 1 ml aliquot (TriCarb 2900, Packard BioScience, Meriden, CT; 4 ml Ultima Gold cocktail, Perkin Elmer, Waltham, MA).

Dissolved organic carbon (DOC) was collected using 5 cm long samplers (Rhizon CSS, Rhizosphere, Wageningen, Netherlands) inserted vertically into the plant rooting zone (sampled depth range: 2–7 cm). Soil solution was retrieved by attaching a pre-evacuated 12 ml vial which also served as sample container. These vials were replaced every 24 hours and collected solutions kept frozen until analysis. DOC was measured (Dimatoc 2000 TOC analyser, Dimatec Analysentechnik GmbH, Germany) and ^{14}C activity in 1 ml sample quantified by liquid scintillation counting (as described above).

Harvest of microcosms

Microcosms designed for destructive analysis were harvested by cutting plant shoots at soil level. Roots were then separated from the soil and cleaned with water. Hyphal ingrowth bags were separated from soil and frozen until further analysis. The remaining soil was sieved at 2 mm, and

a sample stored at 4°C for microbial biomass determination. Another sample was frozen for total soil ^{14}C analysis. Microcosms designed for autoradiographic imaging were also harvested by cutting shoots at soil level. Then, the remaining microcosm, i.e. the intact soil including the plant root system, was frozen.

Analysis of plant and soil material

Plant shoots and roots were dried at 70°C and weighed (needles and branches of *P. mugo* separately). A subsample of the dried plant material was ground or cut into small pieces (*P. mugo* branches). Subsamples were then combusted (Packard 307 sample oxidiser, Perkin Elmer, Waltham, MA) and the trapped $^{14}CO_2$ activity determined by liquid scintillation counting. Hyphal mesh bags were opened and the content suspended in 250 ml H_2O using a blender. A 50 ml aliquot of the suspension was vacuum-filtered (Whatman 42 filter paper, GE Healthcare, Freiburg, Germany). The filter containing the trapped hyphae was folded, dried at 70°C, combusted in the sample oxidizer and ^{14}C activity quantified as described for plant material. Soil microbial C was determined by chloroform fumigation-extraction (Vance, Brookes & Jenkinson 1987). In brief, a 10 g soil subsample was extracted with 50 ml 0.1 M K_2SO_4 (45 min, table shaker at 150 rpm). A second sample was extracted similarly after fumigation with ethanol-free chloroform. Both extracts were filtered (MN 615 filter paper, Macherey-Nagel AG, Oensingen, Switzerland) and microbial biomass calculated from the total extractable organic C content of fumigated and unfumigated samples (Dimatoc 2000 TOC analyser, Dimatec Analysentechnik GmbH, Germany), assuming an extraction efficiency k_{EC}=0.45 (Wu *et al.* 1990). Soil microbial ^{14}C was determined similarly after analysing the extracts by liquid scintillation counting (1 ml extract in 4 ml UltimaGold cocktail).

^{14}C in total soil organic C, which included fungal hyphae and microbial biomass, was analysed like for plant material, after drying soil at 105°C overnight and sieving through a 1 mm mesh.

Autoradiography of soil sections

The frozen soil columns were freeze-dried and sealed at the bottom with semi-hardened epoxy resin (Laromin C 260, BASF, Ludwigshafen, Germany, mixed at a ratio of 2:3 with Araldite DY 026 SP hardener, Astorit AG, Einsiedeln, Switzerland). After the bottom seal had hardened, the soil core was fully impregnated from the top by adding fresh resin. Trapped air was removed by slowly evacuating the impregnated core in a desiccator to ≈20 kPa and then increasing pressure back to atmospheric levels (Stiehl-Braun *et al.* 2011). The resin was left curing for two days at room temperature and then hardened for 24 hours at 60°C. A 6 mm thick vertical section was cut from the centre of the soil cores using a circular diamond saw. This section was further divided into four quarters which were mounted on glass slides. The surface of these soil samples was ground flat (Discoplan TS diamond cup mill, Struers GmbH, Birmensdorf, Switzerland). Autoradiographic images of the sections were obtained by exposing phosphor imaging plates (BAS III S, Fujifilm, Tokyo, Japan) for five days and scanning at a resolution of 250 μm (Fujix BAS 1000 scanner, Fujifilm, Tokyo, Japan). The images of the four slides were recomposed. Prior to further analysis, the area of the section containing the main root of *P. mugo* was excluded. This step was necessary because the fraction of the highly labelled main root visible in the scan varied depending on where exactly the soil core had been cut. In a first step, the autoradiographic images were assessed visually to detect treatment effects on label distribution. Then, background activities were subtracted and the depth profile of the recorded activity calculated (MATLAB 2012b, MathWorks, Natick, MA).

Statistical analyses

All data were analysed by analysis of variance, fitting linear models reflecting the structure of the experimental design. Data from microcosms containing the same species were averaged per plot prior to analysis (these are pseudoreplicates with respect to the temperature treatment). Plot-level data were analysed using a linear model including block and temperature treatment (aov function of R 3.0, http://www-r-project.org). Hierarchical data (e.g. analysis including both species) were analysed by fitting linear mixed-effects models including plot as random effect (ASReml 3.0, VSNI International, Hempstead, UK). Temperature effects were tested using plot as replicate (n=18), whereas e.g. temperature treatment×species was tested using the plot×species combination as replicate (n=36). In general, data were log-transformed to achieve normal distribution of residuals, and to correct for size differences between plant species (e.g. the null hypothesis underlying tests of species×treatment on the log-scale was that temperature effects are proportionally equal). Shifts in ^{14}C partitioning were tested by analysing the ratios of activities measured in the different components of the plant soil system (e.g. shoots : total plant, or plant : total microcosm). The corresponding models included block and treatment×species as fixed effect, plus plot (block×treatment) as random effect. A significant species×treatment interaction thus indicated a species-specific effect of temperature on the proportional distribution of the label within the microcosm.

In all models, the temperature treatment was fitted as continuous term with three equally-spaced levels, i.e. we tested for linear effects of temperature. This corresponds largely to the achieved soil temperatures (the cooling and warming effects on temperature were approximately equal except for the direction). When data showed a non-linear trend, deviation from linearity was tested by additionally fitting a second-order polynomial. Alternatively, when two temperature treatments showed equal responses and only one treatment differed, a contrast corresponding to

these two groups was fitted. We felt that this approach was more adequate to analyse non-linear temperature effects.

The depth distribution recorded in autoradiographies was analysed by aggregating activities in three depth intervals (0–3.3, 3.4–6.6, 6.7–10.0 cm). Depth-dependent effects were then analysed by fitting mixed-effect models including depth×treatment as fixed effect and depth×plot as random effect.

Results

Soil temperature and moisture

Soil temperature averaged 5.8, 12.7 and 19.2°C in the cooling, control, and warming treatment, respectively (logger data, averaging 3, 7 and 10 cm depth). The treatment effect persisted over time and corresponds to a cooling of 6.8°C and a warming of 6.5°C relative to control plots (Fig. 1). The experimental temperature change decreased towards the surface as the heat energy was added (or withdrawn) at the bottom of the pots (Fig. 2).

Volumetric water contents showed strong temporal dynamics, varying between 12% and 54% throughout the experiment (Fig. 1). Differences in moisture between treatments were comparably negligible (-2.3% water volume in warmed relative to cooled microcosms, $F_{2,10}$=3.40, P=0.07). Soils with *L. alpina* were slightly drier (-2.9% water vol.) than soils with *P. mugo* ($F_{1,15}$=11, P<0.01).

Total C pools

Plant biomass was not affected by the temperature treatments, except for *P. mugo* root biomass which was 29% higher in warmed and control relative to cooled soils ($F_{1,10}$=6.7; P<0.05, Fig. 3). Microbial biomass decreased with increasing temperature, irrespective of plant species ($F_{1,11}$=16,

P<0.01; Fig. 3). DOC concentrations (data not shown) averaged to 51 mg l^{-1}, matching the ranges reported under similar conditions (cfr. Hagedorn et al. 2010), and did not depend on temperature treatments and plant species.

Net ^{14}C uptake

Net amounts of ^{14}C assimilated increased with soil temperature ($F_{1,11}$=51.0, P<0.001), an effect that was more pronounced in microcosms with *P. mugo* than in microcosms with *L. alpina* ($F_{1,16}$=13.2, P<0.01, for species×temperature treatment).

Of the total ≈50 kBq ^{14}C released per microcosm, 5, 13 and 19 kBq were recovered after at the destructive harvest in plant plus soil material of cooled, control and warmed *P. mugo* microcosms, respectively. For *L. alpina*, the corresponding amounts were 13, 18 and 18 kBq. When including ^{14}C in soil respiration, recovered ^{14}C increased to 9, 20 and 27 kBq for *P. mugo*, and 19, 25 and 25 kBq for *L. alpina*. Headspace $^{14}CO_2$ at the end of labelling indicated a net uptake of ≈93% of the $^{14}CO_2$ released. ^{14}C recovery in plants, soil, and soil respiration when the microcosms were destructively harvested five days later indicated that ≈43% of the label was recovered in the microcosms, suggesting that at least 50% of the applied label has been respired by plant shoots between radiolabelling and harvest.

^{14}C labelling of plant and soil fractions

Plant biomass ^{14}C increased linearly with temperature in all fractions except *L. alpina* roots which showed no temperature effect (Table 1, Fig. 4). In soils of *P. mugo* microcosms, ^{14}C amounts in all pools (microbial biomass, hyphae, total soil C) were five times lower in the cooling treatment but did not differ between warming and control treatments (Fig. 4, Table 1). In soils of *L. alpina* microcosms, no temperature effect on ^{14}C activity was found in any of the pools

analysed (Fig. 4, Table 1). ^{14}C activities in collected DOC were below the detection limit of our analytical procedure (<0.5 Bq/ml). We added unlabelled DOC to a known standard to test whether counting efficiency was reduced (quenching), but did not detect such effects. Even if DOC had been labelled to the ^{14}C concentrations found in plant material, the ^{14}C in the analysed solutions would have been at the detection limit of our analysis.

^{14}C partitioning among pools

Partitioning of ^{14}C among shoots and roots in *P. mugo*, quantified as the fraction of total plant ^{14}C recovered in roots, did not depend on temperature. In contrast, the fraction of plant ^{14}C recovered in *L. alpina* roots decreased with temperature (Table 2, $F_{1,11}$=15.2, P<0.01), from 54% in cold soils to 38% in warm soils.

In *P. mugo*, partitioning to soil (expressed relative to the sum of plant and soil ^{14}C) was lower in cooled and warmed than in control soils ($F_{1,10}$=6.9, P<0.05). This non-linear effect resulted from a linear increase of ^{14}C in plants with soil temperature, combined with an increase in soil ^{14}C from cold to control but not from control to warm conditions. No effect on ^{14}C partitioning to soil was found in *L. alpina* microcosms. ^{14}C recovered in fungal hyphae and microbial biomass averaged to 1.6% and 3.4% of total microcosm ^{14}C in *P. mugo* and to 0.3% and 6.4% in *L. alpina* microcosms, with these fractions remaining unaffected by temperature.

Soil respiration

Soil CO_2 efflux increased with soil temperature for both species, both prior ($F_{1,11}$=6.9, P<0.001; data not shown) and following label application ($F_{1,9}$=14, P<0.01; Fig. 5 dashed line).

^{14}C activity in soil respiration increased non-linearly with temperature in both plant species ($F_{1,8}$=10.5, P=0.01). In this light, increases in $^{14}CO_2$ efflux appeared particularly large when

moving from cold to control conditions, and increased comparably little from control to warm conditions (Fig. 5, solid line). Relative to the ambient treatment, 84% and 26% less $^{14}CO_2$ were respired in the cooling treatment for *P. mugo* and *L. alpina*, respectively.

Soil $^{14}CO_2$ efflux decreased over the four days following radiolabelling ($F_{1,26}$=109, P<0.001, Fig. 6). The decline of soil $^{14}CO_2$ efflux over the four days following radiolabelling was estimated as first order rate constant in an exponential decay model and did not vary among treatments.

Autoradiographic images

Total ^{14}C activity in autoradiographies (Fig 7) depended on temperature and species (species×temperature: $F_{1,14}$=7.9, P=0.01). Consistent with findings for root and soil ^{14}C activities, total ^{14}C activity increased with soil temperature in *P. mugo* ($F_{1,12}$=14.8, P<0.01), but not in *L. alpina*. ^{14}C distribution along the soil profile depended on the combination of temperature and plant species (species×temperature×depth: $F_{1,64}$=10.8, P<0.002; Fig 7). The shallow *L. alpina* rooting system (reaching 6 to 8 cm depth) resulted in a reduced ^{14}C activity in the lower part of the pot not reached by roots.

When species were analysed separately, ^{14}C in *P. mugo* soils increased with temperature and this effect was larger in the deep soil (depth: $F_{1,32}$=6.9, P=0.01; depth×treatment: $F_{1,32}$=4.6, P<0.05). In contrast, temperature affected ^{14}C distribution over the soil profile in *L. alpina* (depth×treatment: $F_{1,32}$=6.8, P=0.01), but total ^{14}C remained unaffected.

Discussion

Our results demonstrate that, under cold conditions, soil temperature profoundly affects the cycling of new assimilates in the plant-soil system even when air temperature is not manipulated. Net C assimilation strongly increased with soil temperature, and the partitioning of these new assimilates differed between plant species and showed both linear and non-linear responses. The increase in recently fixed C with temperature suggests higher net rates of photosynthesis. However, a direct effect of temperature on photosynthesis (Farquhar, von Caemmerer & Berry 1980; Turnbull, Murthy & Griffin 2002; Grace 2002) can be excluded because air temperature did not vary among treatments. Indirect effects mediated by soil moisture (e.g. via stomatal conductance, Delucia et al. 1991) also appear unlikely since moisture never dropped below 23% of water holding capacity and was only marginally affected by our soil temperature manipulations. Photosynthesis is sensitive to the rate of utilisation and exports of its products (Stitt, Huber & Kerr 1987); therefore, lower C assimilation under cold conditions likely resulted from reduced C sink activities. Such a sink limitation is supported by increased starch concentrations frequently found in plant tissues under cold conditions (Domisch, Finer & Lehto 2001; Kontunen-Soppela *et al.* 2002; Hoch & Körner 2009; Streit *et al.* 2013). This accumulation of photosynthates most likely results from cold-inhibited root growth which abruptly ceases when temperatures drop below a certain threshold, probably around 6°C (Alvarez-Uria & Körner 2007; Hoch & Körner 2009). Although we did not measure starch concentrations, starch accumulation at low temperatures appears to be a fairly general phenomenon. Cold inhibition of root growth or metabolism may therefore explain decreases of net C assimilation with temperature in our study. In line with this presumption, root biomass was lower under soil cooling, at least in one of the species.

Increased ^{14}C uptake at higher temperature could also result from an increased mineral nutrient availability due to accelerated organic matter mineralisation (Melillo *et al.* 2011; Dawes *et al.* 2011). However, nutrients supply to plants was presumably high relative to field conditions even in the cold treatment, because soil sieving likely increased nutrient availability. Plants generally respond to warming by accelerating their development (Arft, Walker & Gurevitch 1999; Domisch *et al.* 2001). Our plants were exposed to manipulated temperatures for approximately seven weeks before labelling and may therefore have been labelled at phenological stages differing in their potential for ^{14}C uptake. Such ontogeny-related effects appear more likely in the deciduous *L. alpina* than in the evergreen *P. mugo*. Accelerated development under warming would have resulted in plants closer to senescence and thus assimilating less C. However, the large positive effect of temperature on assimilation suggests that this either did not occur, or that the effect of ontogeny was comparably small. Conversely, the warming effects on C assimilation we observed may be underestimates.

In line with our findings, many experimental warming studies showed increased aboveground productivity (Rustad *et al.* 2001; Hudson, Henry & Cornwell 2011; Melillo *et al.* 2011; Sistla *et al.* 2013). However, only very few studies report effects on biomass partitioning between shoots and roots, and even less is known about the partitioning of recent C assimilates. In our study, the partitioning of recent C assimilates among roots and shoots varied with temperature in *L. alpina* but not in *P. mugo*. In *L. alpina*, the fraction allocated to roots decreased in the warmed soil; however, this relative effect resulted from increased allocation to shoots rather than decreased allocation to roots. This finding is in agreement with the observations of Paradis et al. (2014) that shoot but not root growth of *Betula glandulosa* seedlings responded to tundra warming using open top chambers. In contrast, Virjamo et al. (2014) found that warming increased root to shoot biomass ratios in *Picea abies* seedlings and argued that plants invested extra resources remaining

after completion of seasonal shoot growth into roots. Similarly, Domisch et al. (2001) reported larger belowground C allocation under soil warming in *Pinus sylvestris* seedlings. Drier soils under warming might explain the increased root : shoot biomass in these studies. Similarly to *L. alpina* in our study, warming increased investment into shoots in other cold-adapted forbs (Hollister & Flaherty 2010).

Our tracing of labelled assimilates are compatible with the idea of a strong reduction of root metabolism and the transfer of recent assimilates to the soil around a critical "threshold" temperature. In *P. mugo*, both accumulation of recent assimilates in soil and respiration of these assimilates did not follow the temperature response of root ^{14}C. The labelling of these fractions increased markedly from the cold to the control treatment, but not further to the warmed treatment, indicating that the additional C assimilated was invested in plant growth, either above or belowground. Soil microbial activity is unlikely to have driven the non-linear responses we observed in labelled respiration. Soil microbes can be active in frozen soils (Drotz *et al.* 2010), and their activity generally increases linearly to exponentially with temperature (Pietikäinen, Pettersson & Bååth 2005). This apparently contrasts the decrease in microbial biomass we found under warming; however, decreased soil microbial biomass under warming has previously been reported and was often accompanied by an increase in microbial activity (Domisch *et al.* 2001; Hagedorn *et al.* 2010; Streit *et al.* 2014). Although temperature-dependent (Gavito *et al.* 2005; Heinemeyer *et al.* 2006; Hawkes *et al.* 2008), mycorrhizal activity has been observed at low (near-freezing) temperatures (Moser 1958). In a soil warming study, Pumpanen et al. (2012) found that – while plant growth and respiration increased with warming – mycorrhizal metrics did not change. We therefore believe that a cold-inhibition of mycorrhizal activity is unlikely, and that root activity and rhizodeposition, rather than microbial metabolism, were limited at low temperatures. Our autoradiographic analyses largely confirmed the results from the harvest data.

In *L. alpina*, no significant temperature effects on belowground allocation (soil plus roots) were found. In contrast, temperature effects increased with soil depth in *P. mugo*, mirroring the magnitude of the applied temperature manipulation. Such differences may result from the two species having different life strategies to cope with cold environments: *L. alpina*, with its shallower rooting system can benefit from warmer topsoil temperatures resulting from direct solar radiation. *P. mugo* roots instead need to grow deeper in the soil to ensure the anchorage of the tree and experience therefore colder temperatures.

Soil CO_2 efflux increased linearly with temperature. The temperature response of soil respiration is the sum of responses of root and the microbial respiration fuelled by both recent assimilates and older C sources. In our study, respiration of recent assimilates ($^{14}CO_2$) responded non-linearly, reflecting the temperature threshold below which root activity and exudation are strongly inhibited. Soil respiration depends on substrate supply (Heinemeyer et al. 2007; Moyano et al. 2007, 2008; Subke et al. 2011), and its response to seasonal temperature variations cannot be separated from indirect effects resulting from other temporal variation in e.g. light intensity and plant phenology (Epron *et al.* 2001; Janssens & Pilegaard 2003). Schindlbacher et al. (2009) therefore concluded that only responses measured under experimental warming reflect the true soil respiration sensitivity to warming. Recent studies applying experimental warming agreed on bulk-soil respiration to be more temperature-sensitive than root and rhizosphere respiration (Hartley *et al.* 2007; Vogel *et al.* 2014; Wang *et al.* 2014). Our findings strongly suggest that the temperature sensitivities of root and rhizosphere respiration depend on the temperature range studied: Warming from low temperatures strongly increased the fraction of recent assimilate-derived CO_2 in soil respiration, whereas warming starting at higher temperatures increased mainly the fraction originating from older C sources. In addition, other factors such as phenological stage (Epron *et al.* 2001) or soil organic matter quality (Leifeld & Fuhrer 2005;

Davidson & Janssens 2006) could be determinant for the response of the two soil respiration components.

Some of the observed effects were species-specific (e.g. recent assimilate partitioning) and generally smaller in *L. alpina*. This may be related to the shallower rooting system of *L. alpina*. The temperature changes induced by our treatment decreased towards the soil surface, so that smaller temperature changes may have been experienced by *L. alpina* roots. This may explain the absence of effects on *L. alpina* root biomass and the absence of threshold-type effects on net soil input of recent assimilates. Nevertheless, effects on belowground C allocation were evident in soil $^{14}CO_2$ efflux data. Warming effects are often species-specific (Hollister & Flaherty 2010; Hudson *et al.* 2011; Dawes *et al.* 2011; Pumpanen *et al.* 2012), but these differences were found to be unrelated to plant functional types in a meta-analysis (Dormann & Woodin 2002). Our results indicate the possibility that such patterns are related to differences in the magnitude of temperature changes, e.g. due to root architecture.

Temperature effects on C allocation depend on the time over which recent assimilates are traced. Our autoradiographies showed that amounts and distribution of label did not change significantly between two and five days. Further, the temporal dynamics of recent assimilates in soil respiration indicated that their turnover rate did not depend on temperature. All of this suggests that the label distribution we found is representative of short-term (days to week) allocation patterns.

In our study, soil temperature treatments were in place for almost two months prior to labelling, i.e. for almost one growing season. In the longer term, warming effects on C dynamics will also depend on factors we did not investigate, including aboveground biomass turnover and changes in vegetation composition (Walker *et al.* 2006; Myers-Smith *et al.* 2011; Sistla *et al.* 2013; Hagedorn *et al.* 2014). Our experiment suggests that rhizodeposition did not increase with

warming, despite increased net ^{14}C assimilation. These disproportionate effects have important implications for soil C storage because turnover rates of rhizodeposits and plant biomass differ (Chapin *et al.* 2009).

In conclusion, our study indicates that warming effects on C cycling strongly depend on the temperature range in which warming occurs. Given the discontinuity of this response, temperature effects on C cycling under cold conditions cannot be predicted based on effects observed under warmer conditions. Our findings further indicate that the critical mechanisms limiting plant growth in cold conditions is effective belowground and likely related to reduced root activity. We therefore speculate that climate change effects on cold ecosystems are driven more strongly by belowground than by aboveground temperatures. This has important implications for the modelling of C cycling under future climatic scenarios, in particular because different changes in air and soil temperature are predicted in a future climate (Jungqvist *et al.* 2014).

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Tables

Table 1 Statistical analyses of soil temperature effects on plant biomass and ^{14}C activity in plant and soil fractions, separate for the two plant species. Effects were tested by linear regression of the response variables against soil temperature (columns named "linear"), and using contrasts comparing cooling treatment with the average effect in warming and control treatments (columns names "threshold"). Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

			P. mugo				*L. alpina*			
Pool	Fraction		linear		threshold		linear		threshold	
			$F_{1,10}$		$F_{1,10}$		$F_{1,10}$		$F_{1,10}$	
C	Plant	Shoot	0.75		1.06		0.10		0.50	
		Root	4.65	.	6.75	*	1.73		0.35	
		Wood	0.36		0.59		-		-	
^{14}C	Plant	Shoot	28.5	***	16.8	**	12.4	**	11.8	**
		Root	48.0	***	35.0	***	0.05		1.04	
		Wood	25.8	***	17.1	**	-		-	
^{14}C	Soil	Microbes	6.29	*	10.2	**	0.001		0.19	
		Fungal hyphae	6.24	*	13.4	**	1.45		0.62	
		SOM	4.63	.	8.24	*	0.01		0.05	

Table 2 Percentage of ^{14}C recovered in plant and soil pools, in dependence of soil temperature treatments.

Plant species	*P. mugo*				*L. alpina*			
Soil Treatment	cold	control	warm	mean	cold	control	warm	mean
Roots	30.7	32.6	35.7	33.0	41.6	40.3	30.3	37.4
Shoots	55.8	40.9	49.3	48.7	35.4	40.5	49.9	41.9
Microbes	2.9	4.5	2.8	3.4	7.2	6.6	5.5	6.4
Fungal hyphae	0.7	2.7	1.4	1.6	0.3	0.4	0.2	0.3
SOM	9.9	19.3	10.9	13.3	15.5	12.2	14.1	13.9

Figures

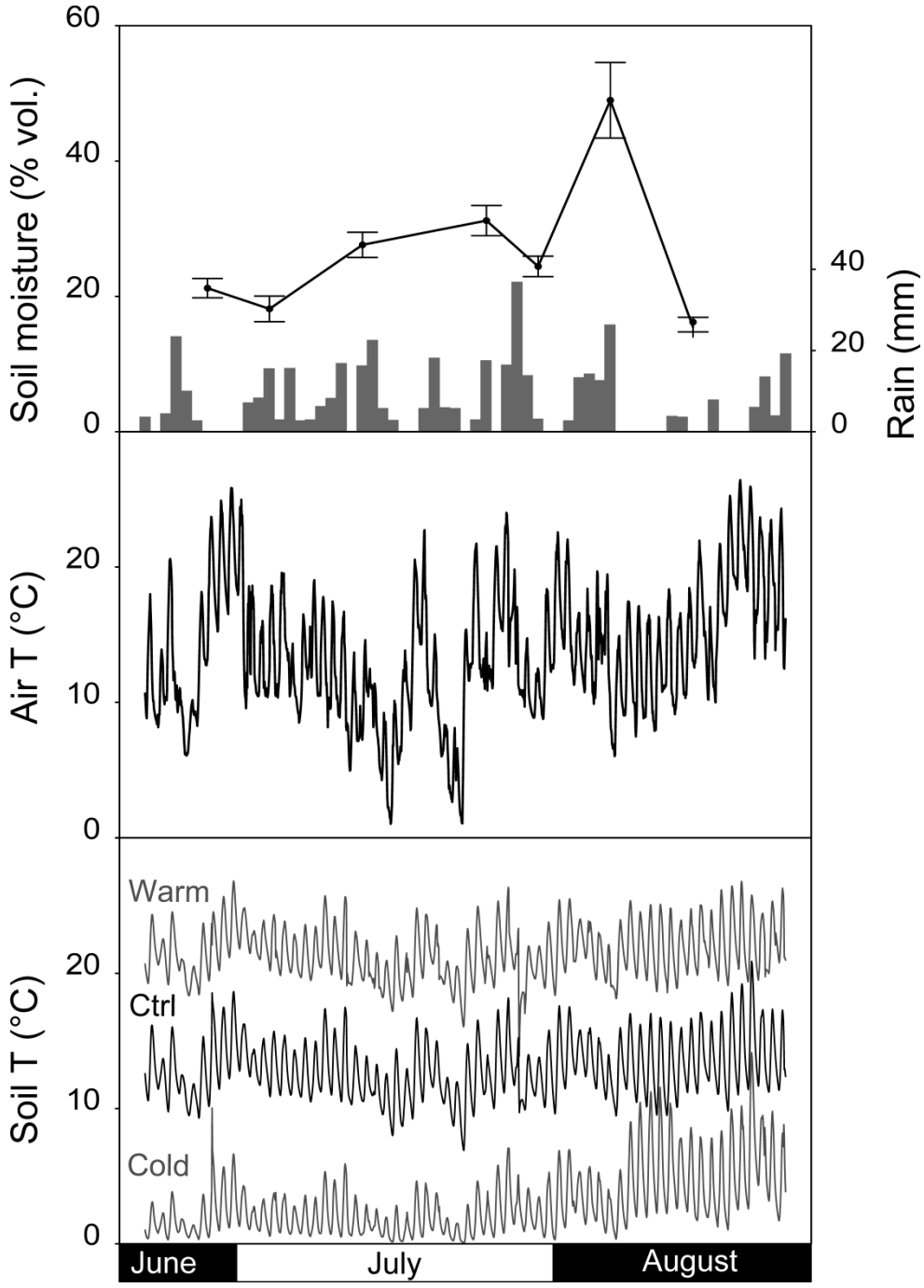

Fig. 1 Volumetric soil water content, precipitation, air and soil temperature over the 57 days of temperature manipulation. Volumetric soil water content was averaged over species and treatments. Error bars show variation among blocks (n=6). Soil water holding capacity is ≈55% vol. Air and soil (10 cm depth) temperatures are averaged hourly and among blocks. Shaded: period of $^{14}CO_2$-pulse-labelling

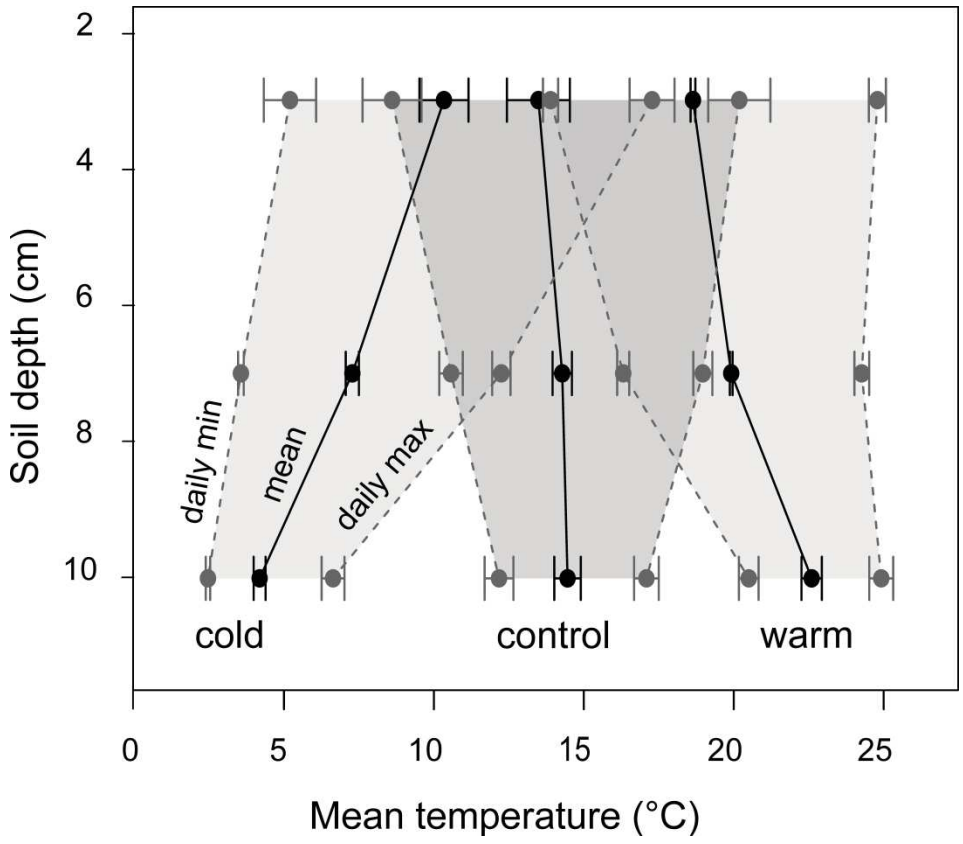

Fig. 2 Daily mean, daily minimum and daily maximum temperature over the soil profile. Error bars are standard errors with block as replicate (n=6)

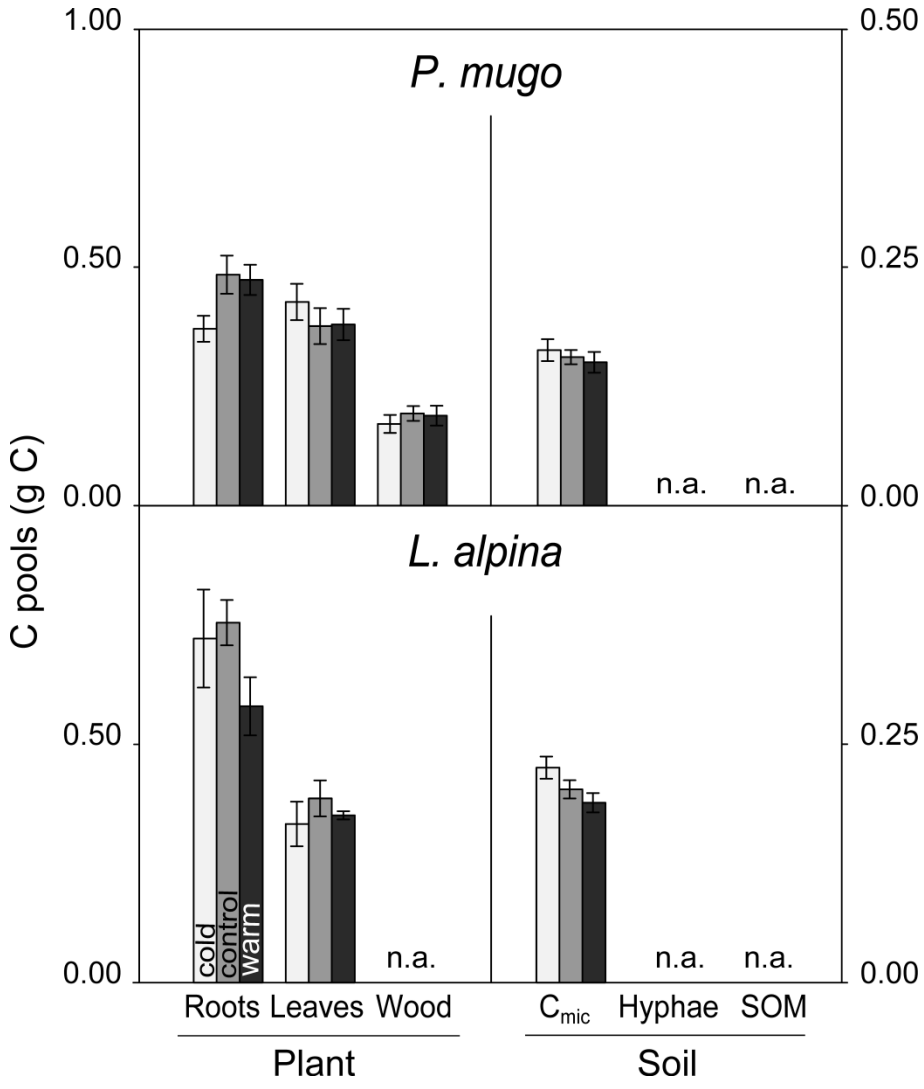

Fig. 3 C pools in microcosms planted with *P. mugo* and *L. alpina.* Soil temperatures averaged 5.9 (cold), 12.7 (control) and 19.2°C (warm). Plant C pools were calculated from biomass assuming a C concentration of 50%. C in fungal hyphae and total soil organic C were not quantified (n.a.). Error bars indicate standard errors across blocks (n=6)

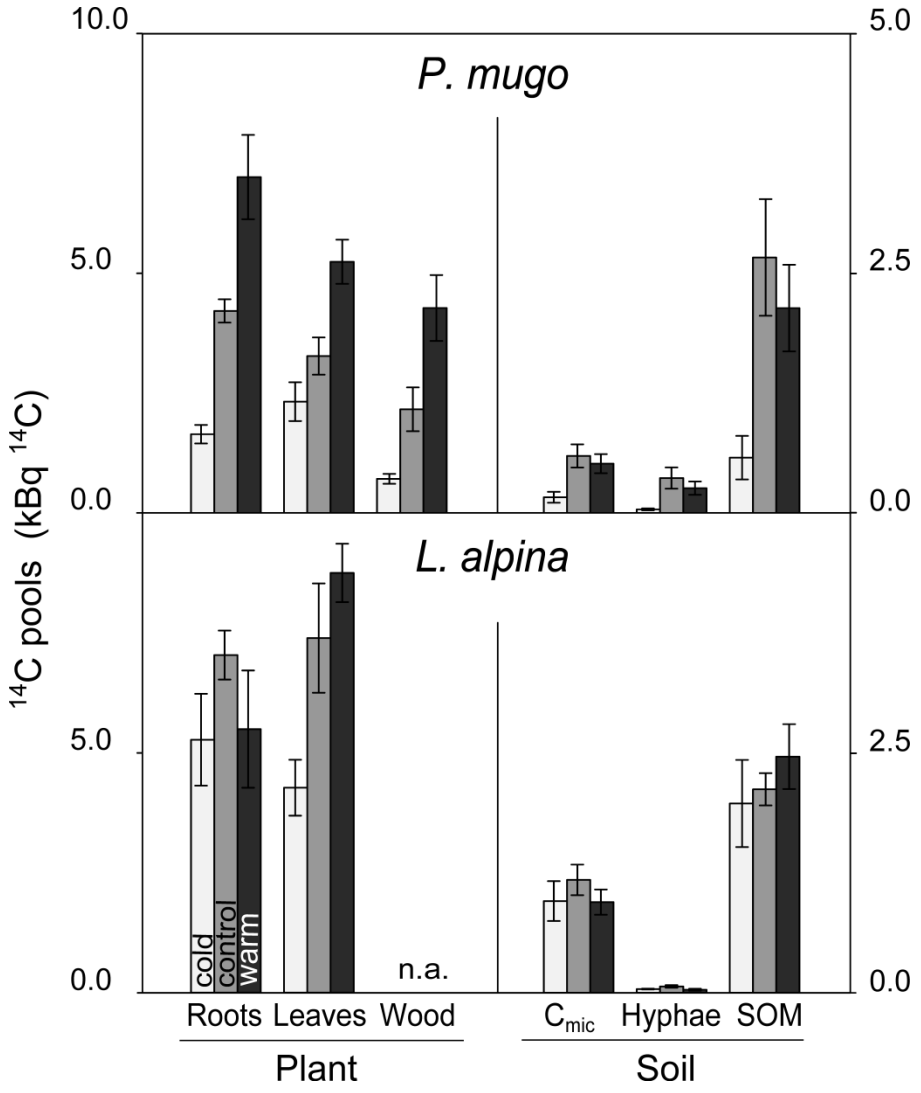

Fig. 4 Soil temperature effects on ^{14}C distribution among plant and soil C pools for microcosms planted with *P. mugo* and *L. alpina* . Note that ^{14}C activities in soil organic matter includes microbial biomass and fungal hyphae. Error bars indicate standard errors across blocks (n=6)

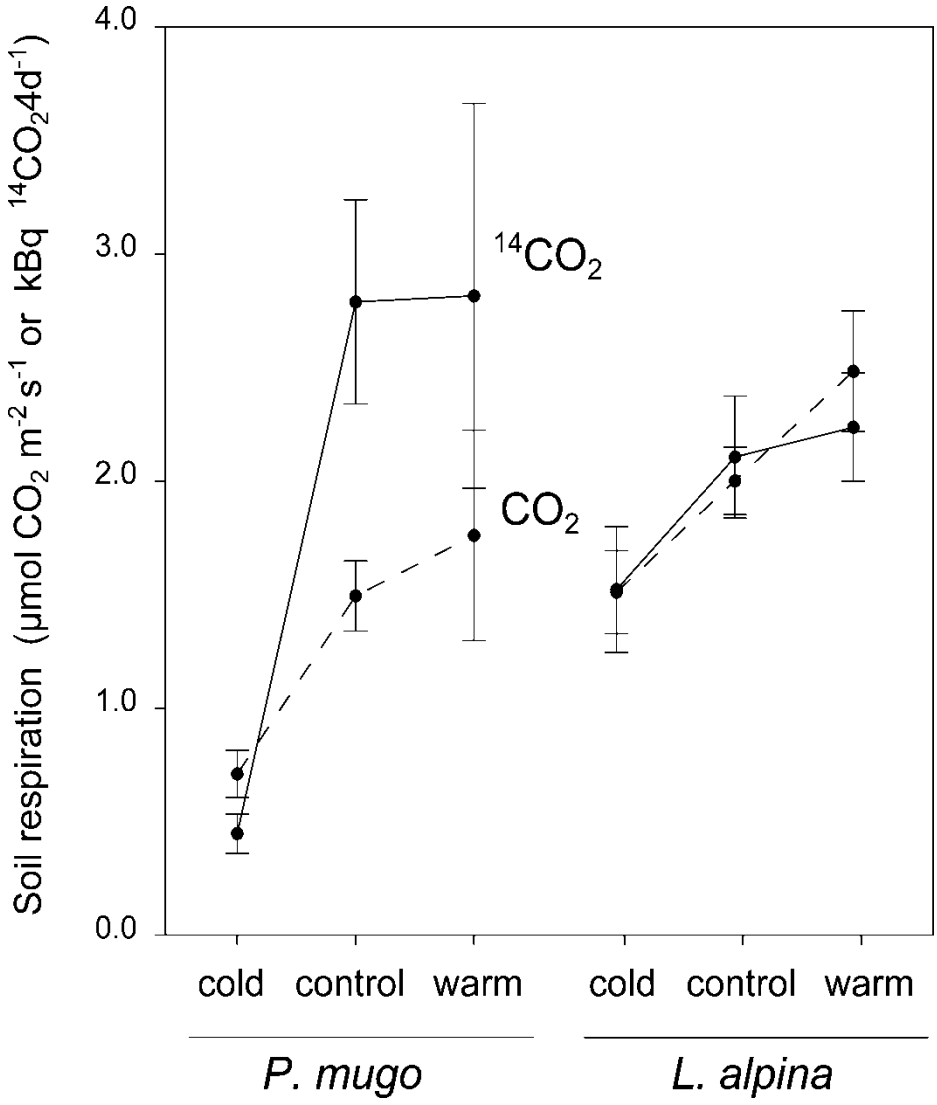

Fig. 5 Soil temperature effects on soil CO_2 and $^{14}CO_2$ efflux. CO_2 efflux is averaged over the day in which radio-labelling occurred and the four following days. $^{14}CO_2$ data are cumulated activities over the four days following the day in which labelling with ^{14}C occurred. All data are on a per microcosm basis, and error bars indicate standard errors across blocks (n=5)

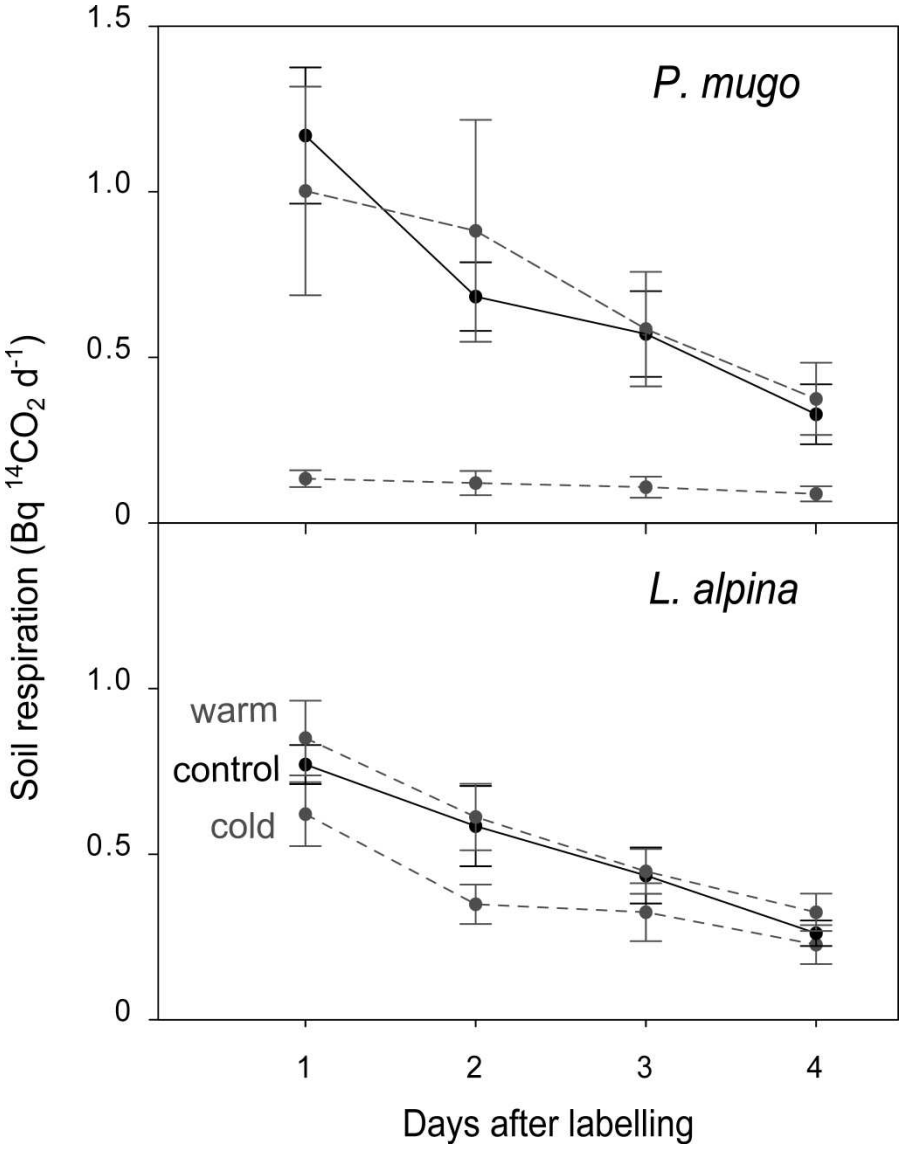

Fig. 6 Soil $^{14}CO_2$ efflux over the 4 days that followed the day of ^{14}C-labelling for *P. mugo* and *L. alpina* exposed to average soil temperatures of 5.9 (cold), 12.7 (control) and 19.2°C (warm). $^{14}CO_2$ efflux for the day of labelling plus the following night is not shown because the NaOH trap likely contained $^{14}CO_2$ label rather than respired $^{14}CO_2$ only. Data are on a per microcosm basis. Error bars indicate standard errors across blocks (n=5, one block removed due to NaOH spill)

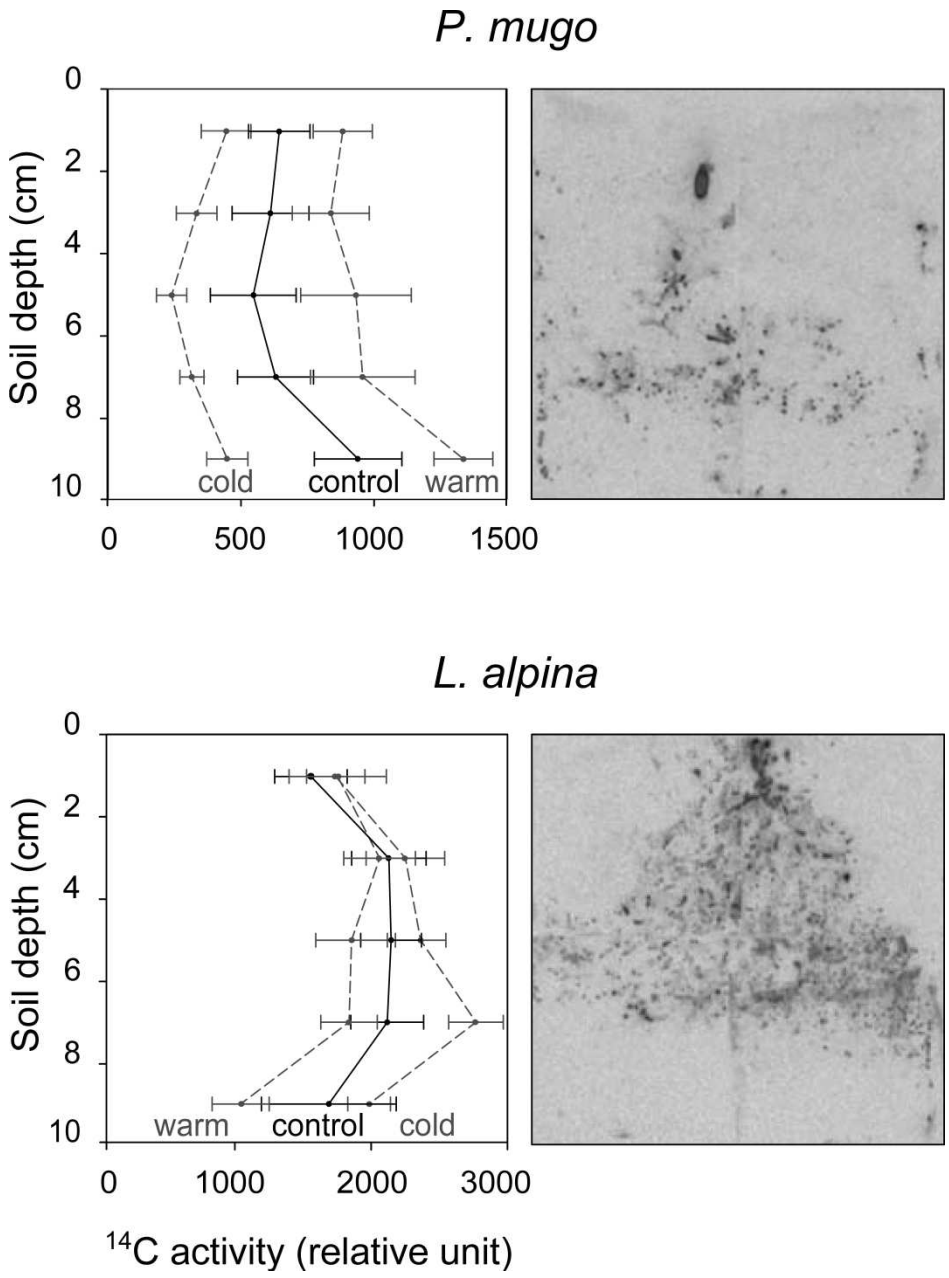

Fig. 7 ^{14}C activity distribution over the 10 cm soil profile in the three soil temperature treatments, at the destructive harvest. Error bars indicate standard errors across blocks (n=6). To the right, an example of an autoradiography for each species is shown, on the same depth scale. Darker pixels indicate higher ^{14}C activity. Note that ^{14}C activity concentrates in roots and rhizosphere without spreading far from the root.